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First record of expansive *Ceratium* Schrank, 1793 species (Dinophyceae) in Southern Brazil, with notes on their dispersive patterns in Brazilian environments

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ABSTRACT: *Ceratium* Schrank is a planktonic dinoflagellate ubiquitous in temperate and subtropical freshwater environments from Northern Hemisphere. Over the past two decades, *Ceratium* species have been recorded in South American water bodies, with expansive behavior and fast colonization. This study registered *C. furcoides* (Levander) Langhans and *C. hirundinella* (O. F. Müller) Dujardin for the first time in South Brazil. *Ceratium furcoides* was found in samples from States of Paraná and Rio Grande do Sul and *C. hirundinella* occurred only in the southernmost Brazil. No co-occurrence of these species was detected on samples. The morphological variation, as well as the dispersal patterns of these species in Brazilian environments, is discussed based on LM and SEM analyses.

Ceratium Schrank is a freshwater dinoflagellate genus with only seven species currently recognized (Hickel 1988a; Popovský and Pfiester 1990; Temponeras *et al.* 2000). The features that define the genus include thick thecal plates, an elongated apical horn formed by apical (') plates, 1–3 antapical horns formed by postcingular (''') and antapical (''') plates, and six cingular plates (three in dorsal view), and its known Kofoidian plate formula is Po 4' 5–6" 6c ?S 5–6"' 2''' (Temponeras *et al.* 2000; Carty 2003; Bicudo and Menezes 2006; Gómez *et al.* 2010). Gómez *et al.* (2010) have redefined the *Ceratium* circumscription, which currently comprises only the freshwater species, and have created the new genus *Neoceratium* Gómez, Moreira *et* López-García to accommodate all the marine species related to the former. The main difference between both genera, from a morphological standpoint, is cingular number of plates - five (two in dorsal view) - in *Neoceratium* (Gómez *et al.* 2010). *Neoceratium* is a debated name for not being in complete accordance with Botanical Nomenclature rules (Calado and Huisman 2010; Gómez 2010). However the distinction between *Ceratium* and *Neoceratium* seems to be undoubted, considering morphological and molecular analyses (Gómez *et al.* 2010).

Ceratium is a common bloom-forming genus in lakes and reservoirs during boreal summer in temperate regions (Pollingher 1988; Carty 2003). Despite the non-toxicity of these blooms (Carty 2003), the aggregated smell and taste to the water and oxygen depletion resulting from massive cell collapse can cause economical and landscape impact (Pollingher 1988; Van Ginkel *et al.* 2001; Hart and Wragg 2009).

Ceratium species are stress-tolerant, due to their swimming abilities which enable diurnal vertical migrations to more favorable light and nutrients microhabitats, and resting cysts formation, which germinate in mixing periods. Moreover, their grazing resistance assures survival during strong zooplankton grazing-pressure (Pollingher 1988; Olrik 1994). *Ceratium hirundinella* (O. F. Müller) Dujardin is the ecologically best-known species (Pollingher 1988), though many records of the species are not documented by figures or morphological information and may correspond, fully or partially, to similar species, such as *C. furcoides* (Levander) Langhans, as discussed by Calado and Larsen (1997).

In Brazil, there were no *Ceratium* records until 2000s. Bicudo and Menezes (2006) cited *Ceratium* with basis on a publication in 1963. However, this paper presented a list of freshwater algae genera with sanitary importance, but not necessary that found in Brazilian waters (Branco *et al*. 1963). This mention can not be taken as a citation of *Ceratium* for Brazil. Moreover, for about 50 years, no other report of this conspicuous alga was made. Since 2003, *C. furcoides* and *C. hirundinella* have been found in several Brazilian aquatic systems. Ferrareze and Nogueira (2006) documented the occurrence of *C. hirundinella* in Paranapanema river basin, São Paulo. *Ceratium furcoides* was registered for the first time in Furnas reservoir, Minas Gerais, by Santos-Wisniewski *et al.* (2007), and more recently in Billings reservoir, São Paulo, by Matsumura-Tundisi *et al.* (2010). Finally, *C. furcoides* and *Ceratium* cf. *hirundinella* were detected by Oliveira *et al.* (2011) in two Brazilian northeastern semiarid basins.

This study documents the first record of *Ceratium* in South Brazil, including a preliminary analysis on dispersal patterns of *C. furcoides* and *C. hirundinella* in Brazilian environments.

Paraná (PR) samples are derived from phytoplankton monitoring program performed by Companhia Paranaense de Energia (COPEL) in hydroelectric power plants (HPP). Rio Grande do Sul (RS) samples are originated from phytoplankton monitoring in surface watersheds to water harvesting for human supplies, performed by Companhia Riograndense de Saneamento (CORSAN). Samplings were conducted weekly to quarterly (Table 1). Subsurface water samples (1L volume) were collected with Van Dorn bottle and preserved with acetic Lugol's solution. *Ceratium* spp. cell densities were estimated by the monitoring teams and were granted for this study by the responsible companies. For qualitative analysis, subsamples were concentered by simple settling or centrifugation and mounted on slides analyzed using an Olympus BX-40 microscope with DP-71 digital camera coupled.

For scanning electron microscopy (SEM), subsamples were washed with distilled water, air-dried on stubs and covered with gold by Balser Sputtering/SDC 300 equipment. These samples were observed on Jeol JSM 6360LV electronic microscope (Centro de Microscopia Eletrônica, Universidade Federal do Paraná), at 15 kV and 8 mm work distance. Aliquots were housed in herbarium of Federal University of Paraná (UPCB 75101, 75105, 75118, 75124-75127).

Two *Ceratium* species were observed. *Ceratium furcoides* occurred in Paraná samples (HPP Chopim, HPP Capivari and HPP São Jorge) and in seven sampling sites from Rio Grande do Sul (Jacuí River, Uruguai River, Itá Dam and Maia Filho Dam). *Ceratium hirundinella* was found only in Aceguá Stream Dam, at Rio Grande do Sul. No co-occurrence of these species has been detected until now.

Despite the low densities in relation to total phytoplankton, *Ceratium* cells have continued to appear along the samplings (Table 2).

Ceratium spp. identification was based on classic studies for this genus (Huber-Pestalozzi 1950; Bourrelly 1970; Hickel 1988b; Popovský and Pfiester 1990; Calado and Larsen1997).

Ceratium hirundinella and *C. furcoides* are common and similar species in relation to outline and size, and may be confused. The main feature distinguishing them is regarding the shape and length of 4' plate, which reaches the apical horn apex in *C. hirundinella* and is shortened in *C. furcoides* (Hickel 1988b; Popovský and Pfiester 1990; Calado and Larsen 1997). In this study, we were able to observe the short 4' in all populations identified as *C. furcoides* (Figures 9–12).

In addition, Popovský and Pfiester (1990) and Hickel (1988b) noted that the epitheca of *C. hirundinella* is bell-shaped, forming an abrupt "shoulder" in apical horn base, whereas the epitheca of *C. furcoides* is conical, with attenuated apical horn, projected forward. This feature could be clearly observed here (compare Figures 1–14 to Figures 15–25). Samples with *C. furcoides* occurrence had no apical horns morphologically similar to *C. hirundinella*. This feature is an important information to practical monitoring purposes, since the epithecal plates shape are not usually noticeable using common cell count techniques.

The hipotheca of both species is quite variable. Bourrelly (1970) pointed out that size and number of horns vary according to environmental changes, especially temperature. In our study, the single *C. hirundinella* population possesses two long antapical horns, being the right one always shorter than the left. In *C. furcoides* two antapical horns were often found: straight (Figure 4) or slightly directed to the right (Figures 1, 2 e 6). However three antapical horns were observed in some specimens,

Table 1. Location of sampling sites, system types and periodicity of sampling.

SAMPLING SITES	HYDROGRAPHIC BASIN	LOCATION	GEOGRAPHIC COORDINATES	SAMPLING PERIODICITY
HPP Capivari	Capivari River Basin	Bocaiúva do Sul, PR	25°08'25"S, 48°52'19"W	quarterly
HPP Chopim	Chopim River Basin	Itapejara D'Oeste, PR	25°59'18"S, 52°44'45"W	quarterly
HPP São Jorge	Pitangui River Basin	Ponta Grossa, PR	25°01'04"S, 50°03'38"W	quarterly
Itá Dam	Apuaê-Inhandava Basin	Marcelino Ramos, RS	27°27'53"S, 51°54'06"W	monthly; weekly
Maia Filho Dam	Upper Jacuí Basin	Salto do Jacuí, RS	29°04'46"S, 53°13'31"W	monthly
Jacuí River	Lower Jacuí Basin	Dona Francisca, RS	29°37'30"S, 53°20'56"W	monthly
Jacuí River	Lower Jacuí Basin	Rio Pardo, RS	29°59'43"S, 52°22'38"W	quarterly
Jacuí River	Lower Jacuí Basin	Cachoeira do Sul, RS	30°03'53"S, 52°53'53"W	monthly
Uruguai River	Butuí-Icamaquã Basin	São Borja, RS	28°37'26"S, 56°02'13"W	monthly
Uruguai River	Ibicuí River Basin	Itaqui, RS	29°06'59"S, 56°32'35"W	monthly
Aceguá Stream Dam	Mirim-São Gonçalo Basin	Aceguá, RS	31°52'07"S, 54°08'53"W	bi-monthly

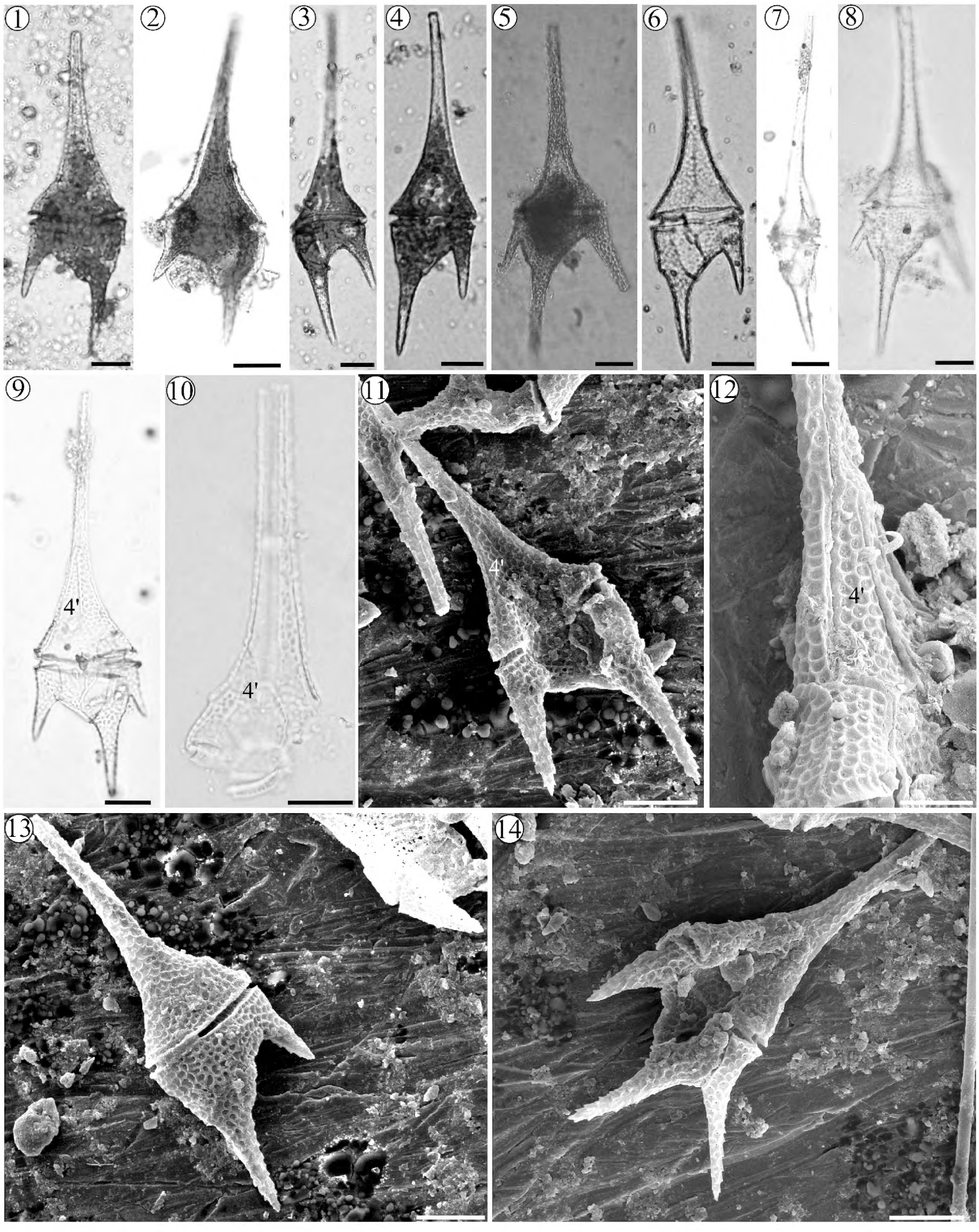
Table 2. Occurrence and relative density of *Ceratium* species in the sampling sites

SAMPLING SITES	OCCURRENCE IN THE SAMPLES	DENSITY IN RELATION TO TOTAL PHYTOPLANKTON
HPP Capivari	Jan, Apr and Jul 2012	0.5 – 11.3%
HPP Chopim	Jun 2012	0.2%
HPP São Jorge	Apr 2012	not found in quantitative analysis
Itá Dam	May 2012	not found in quantitative analysis
Maia Filho Dam	Feb-Jul 2012	0.5 – 5.5%
Jacuí River, Cachoeira do Sul	Apr and Jul 2012	0.4 – 0.6%
Jacuí River, Dona Francisca	Mar-Jul 2012	0.4 – 0.9%
Jacuí River, Rio Pardo	Jul 2012	not found in quantitative analysis
Uruguai River, Itaqui	Dec 2011	not found in quantitative analysis
Uruguai River, São Borja	Jul 2012	not found in quantitative analysis
Aceguá Stream Dam	Jan, Mar and May 2012	0 – 7%

the third one (in the left) being formed by 1'" and 2'" plates (Figures 5, 8 and 14). These findings agree with the morphological variation documented by Hickel (1988b).

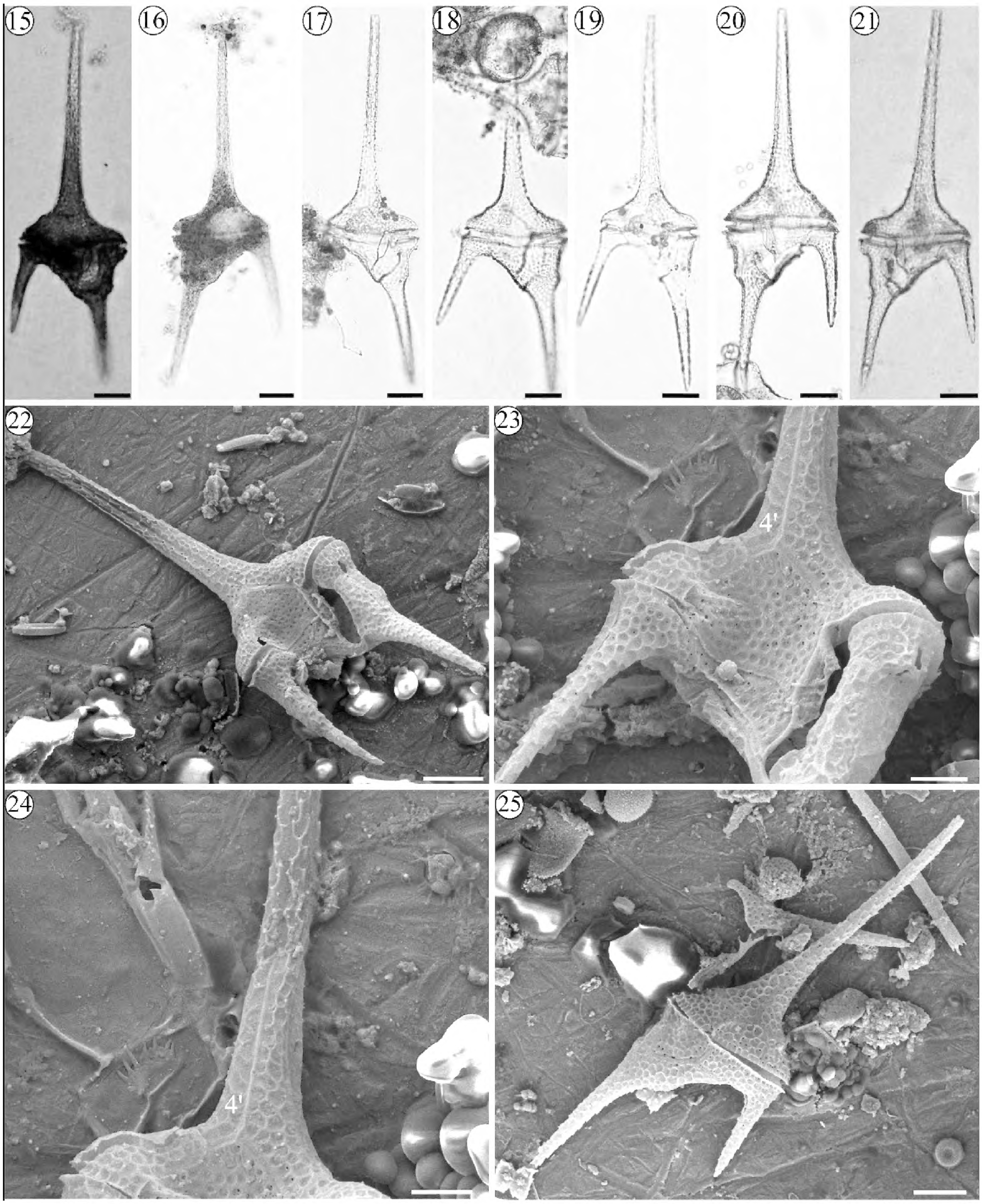
The invasive species in Brazilian environments

Ceratium spp., atypical in Brazilian freshwater systems, have been registered as invasive species in several recent limnological studies performed in Southeast and Northeast region of the country. *Ceratium furcoides* is the main recorded species, always accompanied by illustrations that allow corroborating the identification (Santos-Wisniewski *et al.* 2007; Matsumura-Tundisi *et al.*



Figures 1-14. *Ceratium furcoides.* **1–2.** Cells in ventral view, LM. **3–6.** Cells in dorsal view, LM. **7.** Theca in lateral left view, LM. **8.** Cleaned theca in dorsolateral view, LM. **9–10.** Cleaned epithecal plates showing the shortened 4' plate, LM. **11.** Cell in ventral view, SEM. **12.** Ventral epitheca detail showing the short plate 4', SEM. **13.** Cell in dorsal view. **14.** Cell with three antapical horns, in ventral view, SEM. Scale bars: 10 µm (Figure 12) and 20 µm (Figures 1–11, 13, 14).

2010; Oliveira *et al.* 2011). Conversely, *C. hirundinella* has few unconfirmed records. Ferrareze and Nogueira (2006), in an ecological survey, registered *C. hirundinella*, but no illustrations were added and the study area is located near to that where *C. furcoides* cells have been found. Oliveira *et al.* (2011) reported *Ceratium* cf. *hirundinella,* distinguished from *C. furcoides* specimens in that study only by presence of the third antapical horn. We believe that this taxon also corresponds to *C. furcoides,* considering the conical shape of apical horn from depicted specimen (Oliveira *et al.* 2011, Figure 2D) and the hipothecal variability of *Ceratium* spp. (compare, *e.g.*, with Figure 5 from this study). Based on



Figures 15-25. *Ceratium hirundinella.* **15.** Cell in ventral view, LM. **16.** Cell in dorsal view, LM. **17–19.** Cleaned thecae in ventral view, LM. **20–21.** Cleaned thecae in dorsal view, LM. **22.** Whole cell, in ventral view, SEM. **23.** Detail of ventral epitheca, showing the plate 4', SEM. **24.** Detail of apical horn, showing that 4' reaches the apex, SEM. **25.** Whole cell, in dorsal view, SEM. Scale bars: 10 µm (Figures 23, 24) and 20 µm (Figures 15–22, 25).

the above remarks, it is possible that no *C. hirundinella* has so far been sampled in Brazilian environment. This is therefore the first confirmed record of this species to the country.

Ceratium hirundinella has often been reported to South America since 1990. First recorded in southernmost Argentine lakes, the species quickly established itself in northward Argentina, Chile and Bolivia, particularly in reservoirs (Guerrero and Echenique 1997; Mac Donagh *et al.* 2005; Fontúrbel *et al.* 2006; Silveiro *et al.* 2009). The single confirmed *Ceratium hirundinella* population in this study is from Aceguá, RS, located on the border between Brazil and Uruguay (Table 1), and it is certainly derived from populations that colonized the extreme South of America.

Ceratium furcoides establishment is more recent, and seems to have occurred as a radial dispersion (from southeastern to northwards and southwards). Being an episode in progress, little is known on the dispersal patterns of this flagellate in Brazilian environments. Silva *et al.* (2012) conducted an ecological study about *C. furcoides* in Furnas reservoir, Minas Gerais, and related species abundance with low temperatures and high nutrient concentrations (nitrate and nitrite), although it is considered a perennial dinoflagellate, occurring all the year, even at low densities. Another autecological study in South America involving *C. furcoides* was carried out in Colombia, in which *C. furcoides* abundance were positively related to high chlorophyll a concentration, ammonium, relative water stability column and wind direction, being considered a highly variable species in both temporal and spatial scales (Gil et al. 2012).

Understanding *Ceratium hirundinella* geographical dispersive patterns in Brazil, its co-occurrence with *C. furcoides*, and the controlling factors of distribution and abundance of both species are questions to be elucidated by monitoring of this systems. The tracked study of dispersive behavior of these species, to medium and long term, is a unique opportunity for unveiling the dispersive/establishment mechanisms of freshwater dinoflagellates in neotropical environments.

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